Molecular Biology

A GENETIC AND MOLECULAR ANALYSIS OF THE PROTEIN TAF250

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General transcription factor (GTF) TFIID is one of two sequence specific transcription factors capable of binding to core promoter DNA. The nucleating function of TFIID facilitates transcription initiation by changing chromatin structure to favor RNA polymerase II assembly and by modifying other GTFs. The largest TFIID subunit, TAF250, is one of the multiple TATA-binding protein (TBP)-associated factors (TAFs) complexed with TBP that possesses a number of functions consistent with TFIID activities to regulate transcription initiation. To define and characterize the potential roles of TAF250, we conducted a lethal screen with the goal of isolating novel TAF250 mutants in *Drosophila melanogaster*. We scored 1500 flies and isolated one TAF250 mutant. The TAF250 gene from the mutant fly was sequenced, and a point mutation that converted a glutamine codon to a stop codon was identified. This mutant will be useful for determining the developmental requirements for protein domains located downstream of the stop codon. A second project focused on the C-terminal region (CTR) of TAF250 and its implications in alternative splicing. We cloned sequences homologous to TAF250 from eight *Drosophila* species to identify the presence of two alternatively spliced exons 12a and 13a within the CTR. The cloned DNA sequences were compared to the *Drosophila melanogaster* sequence. One point mutation was identified in exon 12a. This mutation converted a histidine codon to a glutamic acid codon. Two point mutations were identified in exon 13a, changing an alanine codon to threonine and serine to arginine. The high degree of evolutionary conservation of exons 12a and 13a indicate the functional importance of their encoded protein domains. (Supported by the University of Wisconsin Graduate School).